regulated during gastric epithelial cell transformation, and then regains expression in gastric cancer cells undergoing detachment and invasion [53]. In our samples,

The expression status of Caveolin-1 also indicates a tissue-dependent behavior in carcinogenesis. Down-regulation of Caveolin-1 was previously observed in ovarian adenocarcinoma [24], soft tissue sarcoma [56] and thyroid cancer [57]. By contrast, Caveolin-1 was found to be overexpressed in breast and prostate cancer [58], pancreatic adenocarcinoma [59], renal carcinoma [60], lung cancer [61], esophageal carcinoma [62, 63] and colorectal adenocarcinoma [64].

In our sample, Caveolin-1 was expressed in 100% of intestinal type and in 67.9% of diffuse type gastric cancer, hence differing significantly. These findings are also supported by previous reports [53-55], which showed that the intestinal type presented higher expression of Caveolin-1, whereas no or lower immunostaining was found in the diffuse type.

Caveolin-1 usually co-localizes with E-cadherin in plasma membranes [65]. In the present study, we observed that the presence of Caveolin-1 was associated with a lack of E-cadherin expression. This finding corroborates a previous study in hepatocellular carcinoma cells, which demonstrated that Caveolin-1 overexpression leads to a decrease in the E-cadherin expression [66].

The inverse association between E-cadherin and Caveolin-1 may have a stimulant effect for tumor growth through b-catenina pathway [67]. Moreover, the inverse correlation between these proteins reinforces that Caveolin-1 did not have a role as tumor suppressor in our advanced gastric cancer samples. Torres et al [68] showed that the presence of E-cadherin in cancer cells is required for Caveolin-1 functions as a tumor suppressor – for example, leading down-regulation of β-catenin-Tcf/Lef-dependent transcription and Survivin expression.

Helicobacter pylori is the strongest known risk factor for gastric adenocarcinoma. Here, we observed that Caveolin-1 immunoreactivity was more frequent in samples with *H. pylori* infection in diffuse type gastric cancer. In intestinal type gastric cancer, the evaluation of an association between this neoplasia subtype and *H. pylori* was not possible due to a high frequency of

Caveolin-1 expression in these samples. Little is known about the relationship between *H. pylori* and Caveolin-1. Gauthier et al [69] suggested that Caveolin-1 is not necessary to internalize *H. pylori* into cell.

The gene encoding Caveolin-1 (*CAV1*) is located on chromosome 7q31.1 [5]. Thus, the up-regulation of Caveolin-1 observed in the present study may also reflect a chromosome gain of this locus. The trisomy of chromosome 7 was previously observed by our group in gastric cancer cell lines established from tumors and ascitic fluid of individuals from Northern Brazil [70]. Matturri et al [71] observed trisomy of chromosome 7 in 40% of gastric cancer samples. Kokkola et al [72] reported that the most common gains involved chromosome 7 in adenoma, a preneoplastic lesion. Therefore, the frequent detection of trisomy 7 in gastric cancer suggests presence of important oncogenes in this chromosome, such as *CAV1*.

To our knowledge, this is the first study to evaluate *CAV1* methylation in gastric cancer tissue. We observed low frequency of *CAV1* hypermethylation in normal and in tumor gastric tissue. Hence, we did not observe an association between *CAV1* methylation and gastric cancer (Table 2).

In the present study, we used the MSP to evaluate the methylation status of *CAV1*. Although this assay only detects the methylation status at CpG sites within the primer binding sites, it is an interesting methodology to study the methylation pattern of *CAV1*. Cui et al [20] demonstrated that *CAV1* methylation is a generalized event and, thus, the degree of CpG methylation in this gene is not site-specific. Furthermore, the presence of methylated and unmethylated sequences in most of samples through MSP could be due to heterogeneous methylation patterns in different cell populations in gastric samples, as well as all cells presenting methylation at one allele.

Similar to our *CDH1* results, *CAV1* promoter methylation showed high frequency in normal gastric cells (Table 2). Gene promoter methylation is commonly reported in the gastric carcinogenesis process [19, 33, 73-77]. Moreover, it is known that gene methylation status and regulation are frequently tissue-specific or disease-specific [78]. In contrast with gastric cancer, no *CAV1* methylation was observed in urinary bladder adenocarcinoma [79]. In prostate

tumor a hypermethylation of *CAV1* promoter was reported differing from normal tissue [20].

We have also demonstrated an association between methylation status and protein expression, in which hypermethylated tumor samples presented lack of Caveolin-1 expression (Table 3). Epigenetic regulation of *CAV1* by DNA methylation was previously reported in different tumors, such as breast cancer [19, 22], sporadic colorectal cancer tissues [23] and hetapatocellular carcinoma [80].

In conclusion, the methylation status of *CDH1* and *CAV1* was not correlated to gastric carcinogenesis and is probably a common event in gastric mucosa samples of individuals from Northern Brazil. On the other hand, E-cadherin, as described in other populations, is associated with gastric cancer, especially of diffuse type, and with a metastatic phenotype. Caveolin-1 protein and mRNA expression might be a good marker for gastric cancer.

BIBLIOGRAPHY

1. Dowling, P., et al., Proteomic analysis of isolated membrane fractions from superinvasive cancer cells. Biochim Biophys Acta, 2007. 1774(1): p. 93-101.
2. Stemmler, M.P., Cadherins in development and cancer. Mol Biosyst, 2008. 4(8): p. 835-50.
3. Koizume, S., et al., Heterogeneity in the modification and involvement of chromatin components of the CpG island of the silenced human CDH1 gene in cancer cells. Nucleic Acids Res, 2002. 30(21): p. 4770-80.
4. Birchmeier, W. and J. Behrens, Cadherin expression in carcinomas: role in the formation of cell junctions and the prevention of invasiveness. Biochim Biophys Acta, 1994. 1198(1): p. 11-26.
5. Engelman, J.A., et al., Chromosomal localization, genomic organization, and developmental expression of the murine caveolin gene family (Cav-1, -2, and -3). Cav-1 and Cav-2 genes map to a known tumor suppressor locus (6-A2/7q31). FEBS Lett, 1998. 429(3): p. 330-6.
6. Brooks-Wilson, A.R., et al., Germline E-cadherin mutations in hereditary diffuse gastric cancer: assessment of 42 new families and review of genetic screening criteria. J Med Genet, 2004. 41(7): p. 508-17.

7. Guilford, P., et al., E-cadherin germline mutations in familial gastric cancer. Nature, 1998. 392(6674): p. 402-5.
8. Kanai, Y., Alterations of DNA methylation and clinicopathological diversity of human cancers. Pathol Int, 2008. 58(9): p. 544-58.
9. Kangelaris, K.N. and S.B. Gruber, Clinical implications of founder and recurrent CDH1 mutations in hereditary diffuse gastric cancer. Jama, 2007. 297(21): p. 2410-1.
10. Strathdee, G., Epigenetic versus genetic alterations in the inactivation of E-cadherin. Semin Cancer Biol, 2002. 12(5): p. 373-9.
11. Faraldo, M.L., et al., Analysis of the E-cadherin and P-cadherin promoters in murine keratinocyte cell lines from different stages of mouse skin carcinogenesis. Mol Carcinog, 1997. 20(1): p. 33-47.
12. Hennig, G., et al., Progression of carcinoma cells is associated with alterations in chromatin structure and factor binding at the E-cadherin promoter in vivo. Oncogene, 1995. 11(3): p. 475-84.
13. Peinado, H., F. Portillo, and A. Cano, Transcriptional regulation of cadherins during development and carcinogenesis. Int J Dev Biol, 2004. 48(5-6): p. 365-75.
14. Graff, J.R., et al., Methylation patterns of the E-cadherin 5' CpG island are unstable and reflect the dynamic, heterogeneous loss of E-cadherin expression during metastatic progression. J Biol Chem, 2000. 275(4): p. 2727-32.
15. Yoshiura, K., et al., Silencing of the E-cadherin invasion-suppressor gene by CpG methylation in human carcinomas. Proc Natl Acad Sci U S A, 1995. 92(16): p. 7416-9.
16. Goetz, J.G., et al., Caveolin-1 in tumor progression: the good, the bad and the ugly. Cancer Metastasis Rev, 2008. 27(4): p. 715-35.
17. Shin, J., et al., Caveolin-1 is associated with VCAM-1 dependent adhesion of gastric cancer cells to endothelial cells. Cell Physiol Biochem, 2006. 17(5-6): p. 211-20.
18. Razani, B., et al., Caveolin-1, a putative tumour suppressor gene. Biochem Soc Trans, 2001. 29(Pt 4): p. 494-9.
19. Engelman, J.A., X.L. Zhang, and M.P. Lisanti, Sequence and detailed organization of the human caveolin-1 and -2 genes located near the

D7S522 locus (7q31.1). Methylation of a CpG island in the 5' promoter region of the caveolin-1 gene in human breast cancer cell lines. FEBS Lett, 1999. 448(2-3): p. 221-30.

20. Cui, J., et al., Hypermethylation of the caveolin-1 gene promoter in prostate cancer. Prostate, 2001. 46(3): p. 249-56.
21. Bachmann, N., et al., Expression changes of CAV1 and EZH2, located on 7q31 approximately q36, are rarely related to genomic alterations in primary prostate carcinoma. Cancer Genet Cytogenet, 2008. 182(2): p. 103-10.
22. Chen, S.T., et al., Mutational, epigenetic and expressional analyses of caveolin-1 gene in breast cancers. Int J Mol Med, 2004. 14(4): p. 577-82.
23. Lin, S.Y., et al., Promoter CpG methylation of caveolin-1 in sporadic colorectal cancer. Anticancer Res, 2004. 24(3a): p. 1645-50.
24. Wiechen, K., et al., Caveolin-1 is down-regulated in human ovarian carcinoma and acts as a candidate tumor suppressor gene. Am J Pathol, 2001. 159(5): p. 1635-43.
25. Li, W., et al., Expression of the full-length telomerase reverse transcriptase (hTERT) transcript in both malignant and normal gastric tissues. Cancer Lett, 2008. 260(1-2): p. 28-36.
26. Resende, A.L.S., I.E. Matos, and S. Koifman, Mortalidade por câncer gástrico no Estado do Pará, 1980-1997. Arq Gastroenterol, 2006. 43: p. 247-52.
27. Tost, J., DNA Methylation: An Introduction to the Biology and the Disease-Associated Changes of a Promising Biomarker. Mol Biotechnol, 2009.
28. Jones, P.A. and S.B. Baylin, The epigenomics of cancer. Cell, 2007. 128(4): p. 683-92.
29. Batista dos Santos, S.E., et al., Differential contribution of indigenous men and women to the formation of an urban population in the Amazon region as revealed by mtDNA and Y-DNA. Am J Phys Anthropol, 1999. 109(2): p. 175-80.
30. Lauren, P., The Two Histological Main Types of Gastric Carcinoma: Diffuse and So-Called Intestinal-Type Carcinoma. an Attempt at a Histo-Clinical Classification. Acta Pathol Microbiol Scand, 1965. 64: p. 31-49.

31. Sobin, L.H. and C.H. Wittekind, TNM: Classification of malignant tumours. 6th ed2002, New York: Wiley-Liss
32. Herman, J.G., et al., Methylation-specific PCR: a novel PCR assay for methylation status of CpG islands. Proc Natl Acad Sci U S A, 1996. 93(18): p. 9821-6.
33. Leal, M.F., et al., Promoter hypermethylation of CDH1, FHIT, MTAP and PLAGL1 in gastric adenocarcinoma in individuals from Northern Brazil. World J Gastroenterol, 2007. 13(18): p. 2568-74.
34. Liu, Y.C., et al., Mechanisms inactivating the gene for E-cadherin in sporadic gastric carcinomas. World J Gastroenterol, 2006. 12(14): p. 2168-73.
35. Mayer, B., et al., E-cadherin expression in primary and metastatic gastric cancer: down-regulation correlates with cellular dedifferentiation and glandular disintegration. Cancer Res, 1993. 53(7): p. 1690-5.
36. Matsuura, K., et al., Altered expression of E-cadherin in gastric cancer tissues and carcinomatous fluid. Br J Cancer, 1992. 66(6): p. 1122-30.
37. Oka, H., et al., Immunohistochemical evaluation of E-cadherin adhesion molecule expression in human gastric cancer. Virchows Arch A Pathol Anat Histopathol, 1992. 421(2): p. 149-56.
38. Ma, L., et al., miR-9, a MYC/MYCN-activated microRNA, regulates E-cadherin and cancer metastasis. Nat Cell Biol, 2010. 12(3): p. 247-56.
39. Bienz, M. and H. Clevers, Linking colorectal cancer to Wnt signaling. Cell, 2000. 103(2): p. 311-20.
40. Polakis, P., Wnt signaling and cancer. Genes Dev, 2000. 14(15): p. 1837-51.
41. Calcagno, D.Q., et al., MYC and gastric adenocarcinoma carcinogenesis. World J Gastroenterol, 2008. 14(39): p. 5962-8.
42. Calcagno, D.Q., et al., Interrelationship between chromosome 8 aneuploidy, C-MYC amplification and increased expression in individuals from northern Brazil with gastric adenocarcinoma. World J Gastroenterol, 2006. 12(38): p. 6207-11.
43. Calcagno, D.Q., et al., MYC insertions in diffuse-type gastric adenocarcinoma. Anticancer Res, 2009. 29(7): p. 2479-83.

44. Costa Raiol, L.C., et al., Interrelationship between MYC gene numerical aberrations and protein expression in individuals from northern Brazil with early gastric adenocarcinoma. Cancer Genet Cytogenet, 2008. 181(1): p. 31-5.
45. Graziano, F., et al., Combined analysis of E-cadherin gene (CDH1) promoter hypermethylation and E-cadherin protein expression in patients with gastric cancer: implications for treatment with demethylating drugs. Ann Oncol, 2004. 15(3): p. 489-92.
46. Grady, W.M., et al., Methylation of the CDH1 promoter as the second genetic hit in hereditary diffuse gastric cancer. Nat Genet, 2000. 26(1): p. 16-7.
47. Zazula, M., et al., CDH1 gene promoter hypermethylation in gastric cancer: relationship to Goseki grading, microsatellite instability status, and EBV invasion. Diagn Mol Pathol, 2006. 15(1): p. 24-9.
48. Esteller, M., CpG island hypermethylation and tumor suppressor genes: a booming present, a brighter future. Oncogene, 2002. 21(35): p. 5427-40.
49. Silva, P.N., et al., Promoter methylation analysis of SIRT3, SMARCA5, HTERT and CDH1 genes in aging and Alzheimer's disease. J Alzheimers Dis, 2008. 13(2): p. 173-6.
50. Kang, G.H., et al., Aberrant CpG island hypermethylation of chronic gastritis, in relation to aging, gender, intestinal metaplasia, and chronic inflammation. Am J Pathol, 2003. 163(4): p. 1551-6.
51. Issa, J.P., CpG-island methylation in aging and cancer. Curr Top Microbiol Immunol, 2000. 249: p. 101-18.
52. Shen, L., et al., DNA methylation and environmental exposures in human hepatocellular carcinoma. J Natl Cancer Inst, 2002. 94(10): p. 755-61.
53. Burgermeister, E., et al., Differential expression and function of caveolin-1 in human gastric cancer progression. Cancer Res, 2007. 67(18): p. 8519-26.
54. Gao, X., et al., [Down-regulation of caveolin-1 in gastric carcinoma and its clinical biological significance]. Ai Zheng, 2005. 24(3): p. 311-6.

55. Barresi, V., et al., Caveolin-1 immuno-expression in human gastric cancer: histopathogenetic hypotheses. Virchows Arch, 2008. 453(6): p. 571-8.
56. Wiechen, K., et al., Down-regulation of caveolin-1, a candidate tumor suppressor gene, in sarcomas. Am J Pathol, 2001. 158(3): p. 833-9.
57. Aldred, M.A., et al., Caveolin-1 and caveolin-2,together with three bone morphogenetic protein-related genes, may encode novel tumor suppressors down-regulated in sporadic follicular thyroid carcinogenesis. Cancer Res, 2003. 63(11): p. 2864-71.
58. Yang, G., et al., Elevated expression of caveolin is associated with prostate and breast cancer. Clin Cancer Res, 1998. 4(8): p. 1873-80.
59. Suzuoki, M., et al., Impact of caveolin-1 expression on prognosis of pancreatic ductal adenocarcinoma. Br J Cancer, 2002. 87(10): p. 1140-4.
60. Campbell, L., M. Gumbleton, and D.F. Griffiths, Caveolin-1 overexpression predicts poor disease-free survival of patients with clinically confined renal cell carcinoma. Br J Cancer, 2003. 89(10): p. 1909-13.
61. Ho, C.C., et al., Up-regulated caveolin-1 accentuates the metastasis capability of lung adenocarcinoma by inducing filopodia formation. Am J Pathol, 2002. 161(5): p. 1647-56.
62. Ando, T., et al., The overexpression of caveolin-1 and caveolin-2 correlates with a poor prognosis and tumor progression in esophageal squamous cell carcinoma. Oncol Rep, 2007. 18(3): p. 601-9.
63. Kato, K., et al., Overexpression of caveolin-1 in esophageal squamous cell carcinoma correlates with lymph node metastasis and pathologic stage. Cancer, 2002. 94(4): p. 929-33.
64. Fine, S.W., et al., Elevated expression of caveolin-1 in adenocarcinoma of the colon. Am J Clin Pathol, 2001. 115(5): p. 719-24.
65. Galbiati, F., et al., Caveolin-1 expression inhibits Wnt/beta-catenin/Lef-1 signaling by recruiting beta-catenin to caveolae membrane domains. J Biol Chem, 2000. 275(30): p. 23368-77.
66. Cokakli, M., et al., Differential expression of Caveolin-1 in hepatocellular carcinoma: correlation with differentiation state, motility and invasion. BMC Cancer, 2009. 9: p. 65.

67. Selga, E., et al., Role of caveolin 1, E-cadherin, Enolase 2 and PKCalpha on resistance to methotrexate in human HT29 colon cancer cells. BMC Med Genomics, 2008. 1: p. 35.
68. Torres, V.A., et al., E-cadherin is required for caveolin-1-mediated down-regulation of the inhibitor of apoptosis protein survivin via reduced beta-catenin-Tcf/Lef-dependent transcription. Mol Cell Biol, 2007. 27(21): p. 7703-17.
69. Gauthier, N.C., et al., Helicobacter pylori VacA cytotoxin: a probe for a clathrin-independent and Cdc42-dependent pinocytic pathway routed to late endosomes. Mol Biol Cell, 2005. 16(10): p. 4852-66.
70. Leal, M.F., et al., Establishment and conventional cytogenetic characterization of three gastric cancer cell lines. Cancer Genet Cytogenet, 2009. 195(1): p. 85-91.
71. Matturri, L., et al., Prognostic significance of different biological markers (DNA index, PCNA index, apoptosis, p53, karyotype) in 126 adenocarcinoma gastric biopsies. Anticancer Res, 1998. 18(4B): p. 2819-25.
72. Kokkola, A., et al., Presence of high-level DNA copy number gains in gastric carcinoma and severely dysplastic adenomas but not in moderately dysplastic adenomas. Cancer Genet Cytogenet, 1998. 107(1): p. 32-6.
73. Lima, E.M., et al., Study of Methylation Pattern of de Novo DNA Methytransferase Genes and its Correlation with DNA Methylation Pattern of RUNX3 in Individuals with Gastric Cancer from Northern Region of Brazil. Int J Morphol, 2007. 25(4): p. 817-824.
74. Lima, E.M., et al., Methylation status of ANAPC1, CDKN2A and TP53 promoter genes in individuals with gastric cancer. Braz J Med Biol Res, 2008. 41(6): p. 539-43.
75. Moura Lima, E., et al., DNA mismatch repair gene methylation in gastric cancer in individuals from northern Brazil. Biocell, 2008. 32(3): p. 237-43.
76. Gigek, C.O., et al., hTERT methylation and expression in gastric cancer. Biomarkers, 2009. 14(8): p. 630.

77. Gigek, C.O., et al., Insulin-like growth factor binding protein-3 gene methylation and protein expression in gastric adenocarcinoma. Growth Horm IGF Res, 2010. 20(3): p. 234-8.
78. Nagase, H. and S. Ghosh, Epigenetics: differential DNA methylation in mammalian somatic tissues. Febs J, 2008. 275(8): p. 1617-23.
79. Kunze, E. and T. Schlott, High frequency of promoter methylation of the 14-3-3 sigma and CAGE-1 genes, but lack of hypermethylation of the caveolin-1 gene, in primary adenocarcinomas and signet ring cell carcinomas of the urinary bladder. Int J Mol Med, 2007. 20(4): p. 557-63.
80. Hirasawa, Y., et al., Methylation status of genes upregulated by demethylating agent 5-aza-2'-deoxycytidine in hepatocellular carcinoma. Oncology, 2006. 71(1-2): p. 77-85.

Table 1: Clinicopathological characteristics and immunohistochemistry results of gastric tissue samples, n (%).

	E-cadherin				Caveolin-1			
Variable	Total	Positive	Negative	p value	Total	Positive	Negative	p value
Gender								
Male	30	15 (50%)	15 (50%)	p=0.3693	39	33 (84.6%)	6 (15.4%)	p=0.9972
Female	18	6 (33.3%)	12 (66.7%)		18	15 (83.3%)	3 (16.7%)	
Tissue								
NGM	20	20 (100%)	0	p<0.0001*	18	1 (5.6%)	17 (94.4%)	p<0.0001*
GC	58	21 (36.2%)	37 (63.8%)		57	48 (84.2%)	9 (15.8%)	
Onset								
≤ 45y	6	3 (50%)	3 (50%)	p=0.6573	5	4 (80%)	1 (20%)	p=0.9759
> 45y	52	18 (34.6%)	34 (65.4%)		52	44 (84.6%)	8 (15.4%)	
H. pylori								
Present	42	14 (33.3%)	28 (66.7%)	p=0.5460	41	37 (90.2%)	4 (9.8%)	p=0.0987
Absent	16	7 (43.8%)	9 (56.2%)		16	11 (68.8%)	5 (31.2%)	
Laurén Classification								
Diffuse	32	3 (9.4%)	29 (90.6%)	p<0.0001*	28	19 (67.9%)	9 (32.1%)	p<0.0008*
Intestinal	26	18 (69.2%)	8 (30.8%)		29	29 (100%)	0	
Tumor Location								
Cardia	11	7 (63.6%)	4 (36.4%)	p=0.0775	11	9 (81.8%)	2 (18.2%)	p=0.9908
Noncardia	47	14 (29.8%)	33 (70.2%)		46	39 (84.8%)	7 (15.2%)	
Stage								
I/II	5	0	5 (100%)	p=0.1479	4	4 (100%)	0	p=0.6059
III/IV	53	21 (39.6%)	32 (60.4%)		53	44 (83%)	9 (17%)	
Lymph node mestasis								
Present	53	21 (39.6%)	32 (60.4%)	p=0.1479	53	44 (83%)	9 (17%)	p=0.6059
Absent	5	0	5 (100%)		4	4 (100%)	0	
Distant metastasis								
Present	18	12 (66.7%)	6 (33.3%)	p=0.0035*	20	18 (90%)	2 (10%)	p=0.4582
Absent	37	9 (24.3%)	28 (75.7%)		34	27 (79.4%)	7 (20.6%)	
Unkown	3				3			

IHC: immunohistochemistry assay; NGM: normal gastric mucosa; GC: gastric cancer

Table 2: Clinicopathological characteristics methylation frequency in gastric tissue samples, n (%).

Variable	*CDH1*				*CAV1*			
	Total	M n (%)	U n (%)	p value	Total	M n (%)	U n (%)	p value
Gender								
Male	70	64 (91.4%)	6 (8.6%)	p=0.7311	59	19 (32.2%)	40 (67.8%)	p=0.6371
Female	36	32 (88.9%)	4 (11.1%)		29	11 (37.9%)	18 (62.1%)	
Tissue								
NGM	52	47 (90.4%)	5 (9.6%)	p=0.9991	43	12 (27.9%)	31 (72.1%)	p=0.6081
GC	106	96 (90.6%)	10 (9.4%)		88	30 (34.1%)	58 (65.9%)	
Onset								
≤ 45y	14	11 (78.6%)	3 (21.4%)	p=0.1259	10	2 (20%)	8 (80%)	p=0.4838
> 45y	92	85 (92.4%)	7 (7.6%)		78	28 (36.9%)	50 (64.1%)	
H. pylori								
Present	71	66 (93%)	5 (7%)	p=0.2926	59	17 (28.8%)	42 (71.2%)	p=0.0987
Absent	35	30 (85.7%)	5 (14.3%)		29	13 (44.8%)	16 (55.2%)	
Laurén Classification								
Diffuse	55	47 (85.5%)	8 (14.5%)	p=0.1779	44	18 (40.9%)	26 (59.1%)	p=0.2608
Intestinal	51	49 (96%)	2 (4%)		44	12 (27.3%)	32 (72.7%)	
Tumor Location								
Cardia	22	21 (95.5%)	1 (4.5%)	p=1	22	9 (40.9%)	13 (59.1%)	p=0.4372
Noncardia	80	74 (92.5%)	6 (7.5%)		65	20 (30.8%)	45 (69.2%)	
Stage								
I/II	10	9 (90%)	1 (10%)		6	4 (66.7%)	2 (33.3%)	p=0.1746
III/IV	95	86 (90.5%)	9 (9.5%)	p=0.9472	82	26 (31.7%)	56 (68.3%)	
Lymph node mestasis								
Present	92	84 (91.3%)	8 (8.7%)	p=10.6061	80	27 (33.8%)	53 (66.2%)	p=0.9981
Absent	13	11 (84.6%)	2 (15.4%)		8	3 (37.5%)	5 (62.5%)	
Distant metastasis								
Present	30	28 (93.3%)	2 (6.7%)	p=0.5982	27	8 (29.6%)	19 (70.4%)	p=0.8030
Absent	60	58 (96.7%)	2 (3.3%)		54	19 (35.2%)	35 (64.8%)	
Unkown	15				7			

IHC: immunohistochemistry assay; NGM: normal gastric mucosa; GC: gastric cancer

Table 3: *CDH1* and *CAV1* promoter methylation and protein expression in tumor and normal gastric samples.

		CDH1				*CAV1*			
	IHC / MSP	Positive	Negative	N	p	Positive	Negative	N	p
Tumor	M	21 (37.5%)	35 (62.5%)	56	p=1	5 (41.7%)	7 (58.3%)	12	p=0.0001*
	U	0	0	0		42 (95.5%)	2 (5.5%)	44	
Normal	M	20 (100%)	0	20	p=1	0	6 (100%)	6	p=1
	U	0	0	0		1 (9.1%)	10 (90.9%)	11	
Total	M	41 (53.9%)	35 (46.1%)	76	p=1	5 (27.8%)	13 (72.2%)	18	p=0.003*
	U	0	0	0		43 (78.2%)	12 (21.8%)	55	

IHC: immunohistochemistry assay; MSP: methylation specific PCR; M: methylated; U: unmethylated.

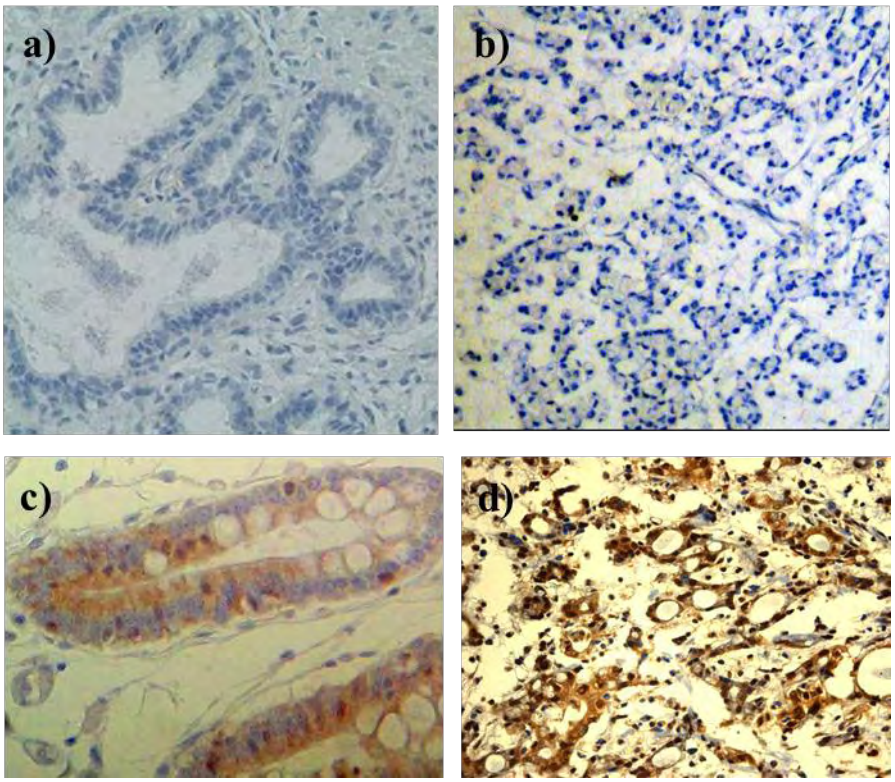

Figure 1: Immunohistochemical detection of proteins. a) no immunoreactity in intestinal type gastric cancer for E-cadherin, b) no immunoreactity in diffuse type gastric cancer for E-cadherin, c) positive immunostaining in intestinal type gastric cancer for Caveolin-1, d) positive immunostaining in diffuse type gastric cancer for E-cadherin. Magnification: 40x.

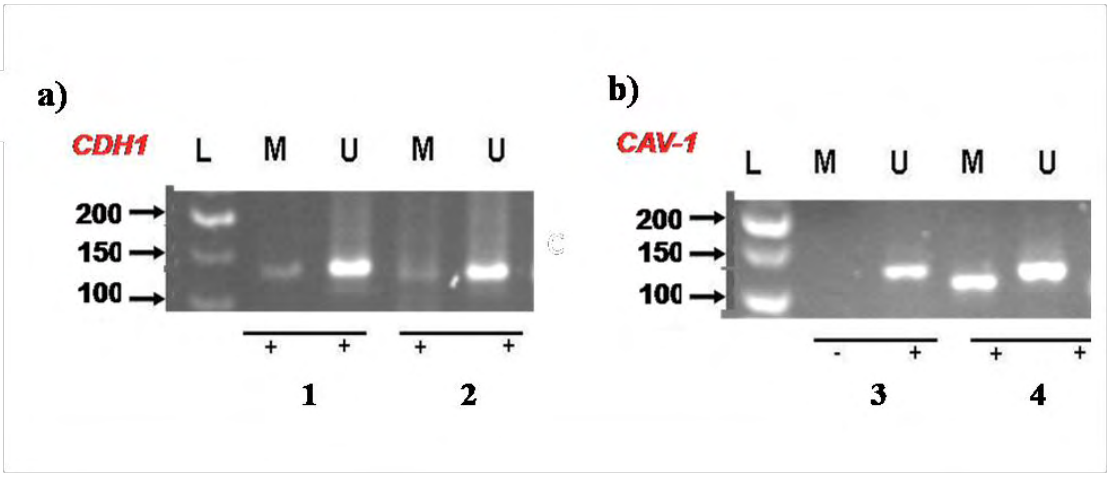

Figure 2: Methylation analysis by MSP. a) *CDH1* reaction showing unmethylated and methylated samples, b) *CAV-1* reaction showing unmethylated and methylated samples. L: size marker; M: methylated; U: unmethylated.

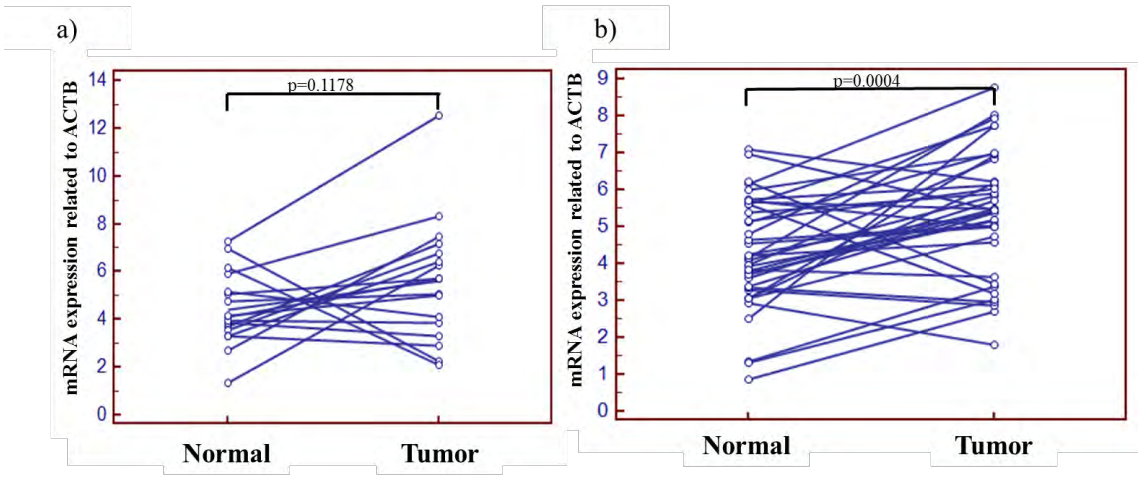

Figure 3: Expression of both genes in gastric samples. a) Quantitative RT-PCR analysis of CDH1 and b) of CAV1 (linear scale).